OBSERVATIONS ON AN ENDAMŒBA PARASITIZING OPALINID CILIATES

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Introduction

Endamœba is well known as a parasite of Metazoa, but it had not been identified as a parasite of Protozoa until 1933, when a brief notice of this remarkable phenomenon was published by Stabler. The protozoans found infected with *Endamœba* were opalinid ciliates, living in the rectum of frogs and toads.

The first observers of endamœbæ within the opalinids either did not recognize them as parasites at all or identified them incorrectly. Thus, Metcalf (1923) described them as "secondary" and degenerating nuclei which he believed to be "abnormal" and "associated with parasitism." Carini (1933*a* and *b*) recognized them as parasites, but thought they represented a new genus which he called *Brumptina*, in honor of Professor Brumpt, who had seen but not identified the same objects in 1913. Since Stabler (1933) recognized these "secondary nuclei" and "*Brumptina*" as endamœbæ, the report has been confirmed by the recent observations of Carini and Reichenow (1935).

In the present contribution, we report further observations on the endamœbæ infecting opalinids from a number of different localities. These observations have been supplemented by independent examination of the original preparations of Metcalf and Carini.

Material and Methods

The material consists of the following opalinids parasitized by amœbæ: *Zelleriella hirsuta* in *Bufo cognatus cognatus* from Flagstaff, Arizona; *Z. opisthocarya* in *B. marinus* from Panama City, Panama; zelleriellas in *B. woodhousii* from Somerton, Arizona and from the vicinity of Tuba City, Arizona; and zelleriellas in *Pleurodema bibroni* from the vicinity of Coquimbo, Chile. A number of other species of anurans and their opalinids from different localities were also examined, but no endamœbæ were found in them.

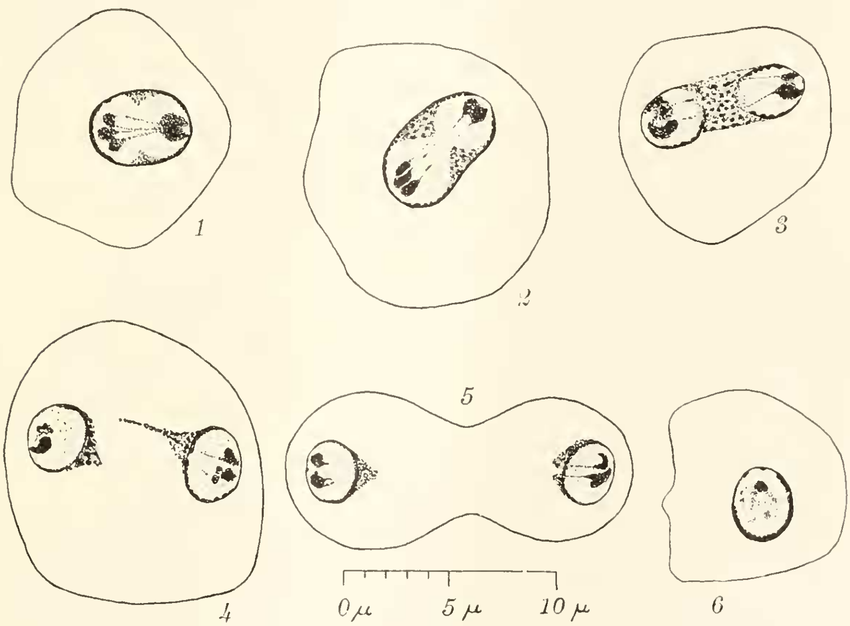
The ciliates containing the parasites were studied to some extent in 0.8 per cent NaCl and in dilute Lugol's iodine solution, but mainly on smears fixed in warm Schaudinn's fluid containing 5 per cent of glacial acetic acid and subsequently stained in Heidenhain's hæmatoxylin. Some of the destaining was carried out in iron alum, some in a saturated aqueous solution of picric acid.

All drawings were made with the aid of the camera lucida.

Original Observations

General Description

The organism found living in the cytoplasm of these opalinids is an *Endamœba*, with all the features characteristic of the genus. It greatly resembles *Endamœba ranarum* morphologically.



Figs. 1–6. Late division stages in the endamœbæ. For explanation see text.

Observed in the living condition, the amœbæ appear as small refractile bodies within the cytoplasm of the actively moving ciliates. Due to their small size, they could easily be overlooked, as the opalinids turn and dart about. In the iodine solution, however, they are easily discernible and their *Endamœba* characteristics readily noted. The amœbæ seem to have little or no effect on the ciliate. Not only do the ciliates move actively about, but they also undergo normal binary fission despite the presence of numbers of amœbæ in their cytoplasm (Fig. 22).

Although a good number of species of anurans and their opalinids from different localities have been examined, only certain species of anurans from some localities and a certain percentage of these latter individuals contain ciliates parasitized by the endamœbæ. Within an individual anuran host, if the opalinids are thus parasitized, a number of these ciliates may still be entirely free from the amœbæ, some harboring a single amœba, others a number of them (Fig. 23) and still others may have their cytoplasm filled with well over a hundred of these parasites (Fig. 24).

The amœbæ are often seen lying in pockets in the cytoplasm of the ciliates and occasionally two or more amœbæ are found in the same pocket (Fig. 10). The space between the amœbæ and the cytoplasm may represent, partially at least, a shrinkage space resulting from fixation and dehydration. A number of amœbæ have been found exerting such pressure on the nuclear membranes of the opalinids that the membranes were markedly indented (Fig. 8). No amœba has ever been observed within a nucleus, however. Usually all the amœbæ in any one ciliate are in the same stage of development, i.e. all cysts or all trophozoites (Figs. 22, 23, and 24).

DESCRIPTION OF PLATES

Drawings all made with the aid of the camera lucida from material stained in Heidenhain's hæmatoxylin and destained in iron alum except where noted.

Explanation of Plate I

Figs. 7–18 are of the *Endamœba* living in the opalinids.

Figs. 19–21 are of *Endamœba ranarum*.

Fig. 7. Trophozoite in the periphery of *Zelleriella* showing ingested endospherules.

Fig. 8. An amœba indenting the nuclear membrane of the ciliate.

Fig. 9. Trophozoite, the nucleus of which shows an abundance of stainable material about the karyosome. Destained in a saturated aqueous solution of picric acid.

Fig. 10. Two amœbæ in the same pocket.

Fig. 11. Two nuclei in the same amœba.

Fig. 12. An amœba outside the opalinids. Note absence of food bodies and the abundance of stainable material in the center of the nucleus. Destained in picric acid.

Fig. 13. Amœba containing the *Sphærita*-like organisms. Destained in picric acid.

Fig. 14. Trophozoite showing the typical *Endamœba* features.

Fig. 15. Typical uninucleate cyst within a *Zelleriella*.

Fig. 16. A very small trophozoite. Destained in picric acid.

Fig. 17. Quadrinucleate cyst outside the ciliates. Note the *E. coli*-like chromatoids.

Fig. 18. Quadrinucleate cyst outside the ciliates. Note the *E. histolytica*-like chromatoid. Destained in picric acid. Compare with Fig. 21.

Fig. 19. *E. ranarum* trophozoite. Note ingested bacteria.

Fig. 20. Uninucleate cyst of *E. ranarum*.

Fig. 21. Quadrinucleate cyst of *E. ranarum*. Compare with Fig. 18.

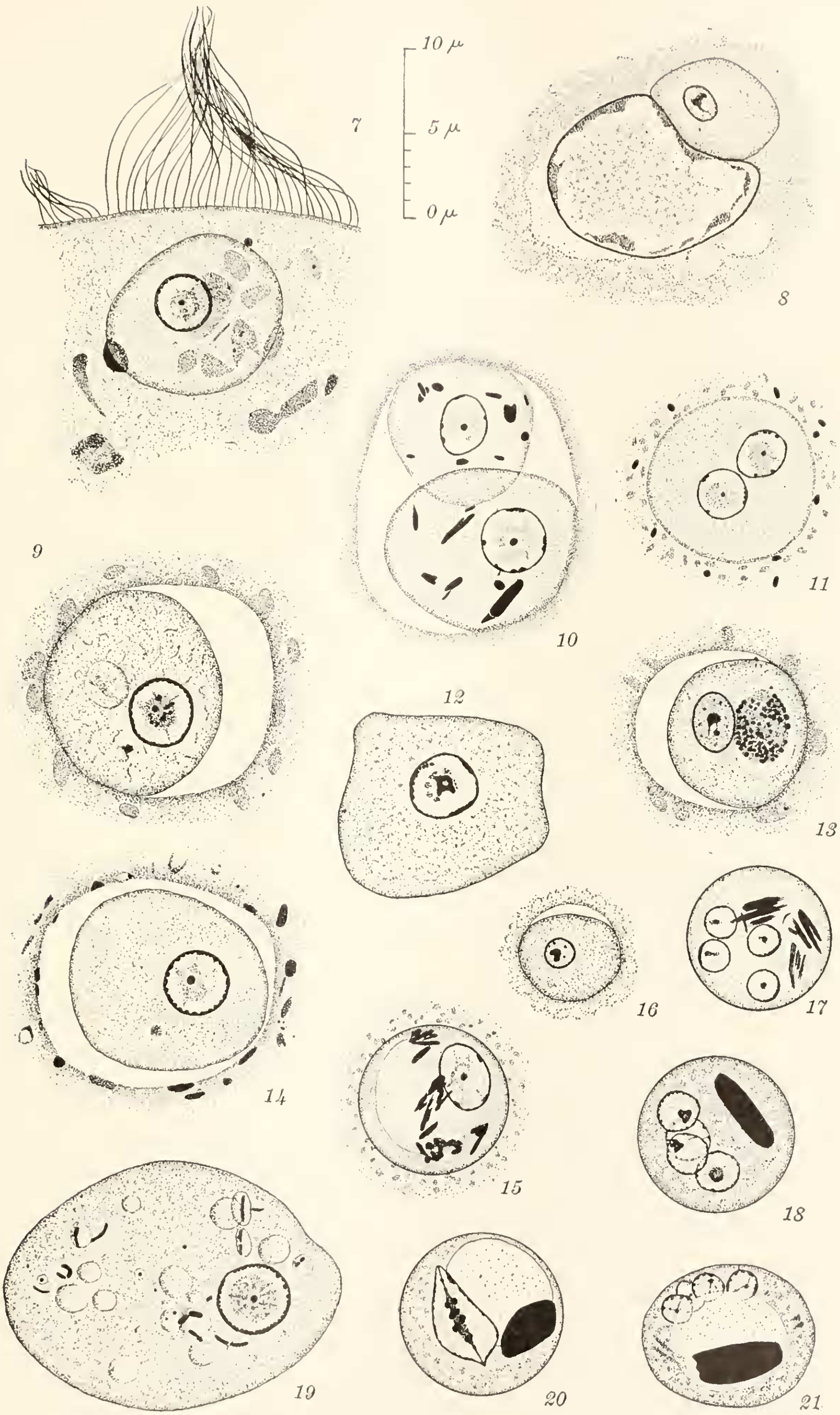


PLATE I

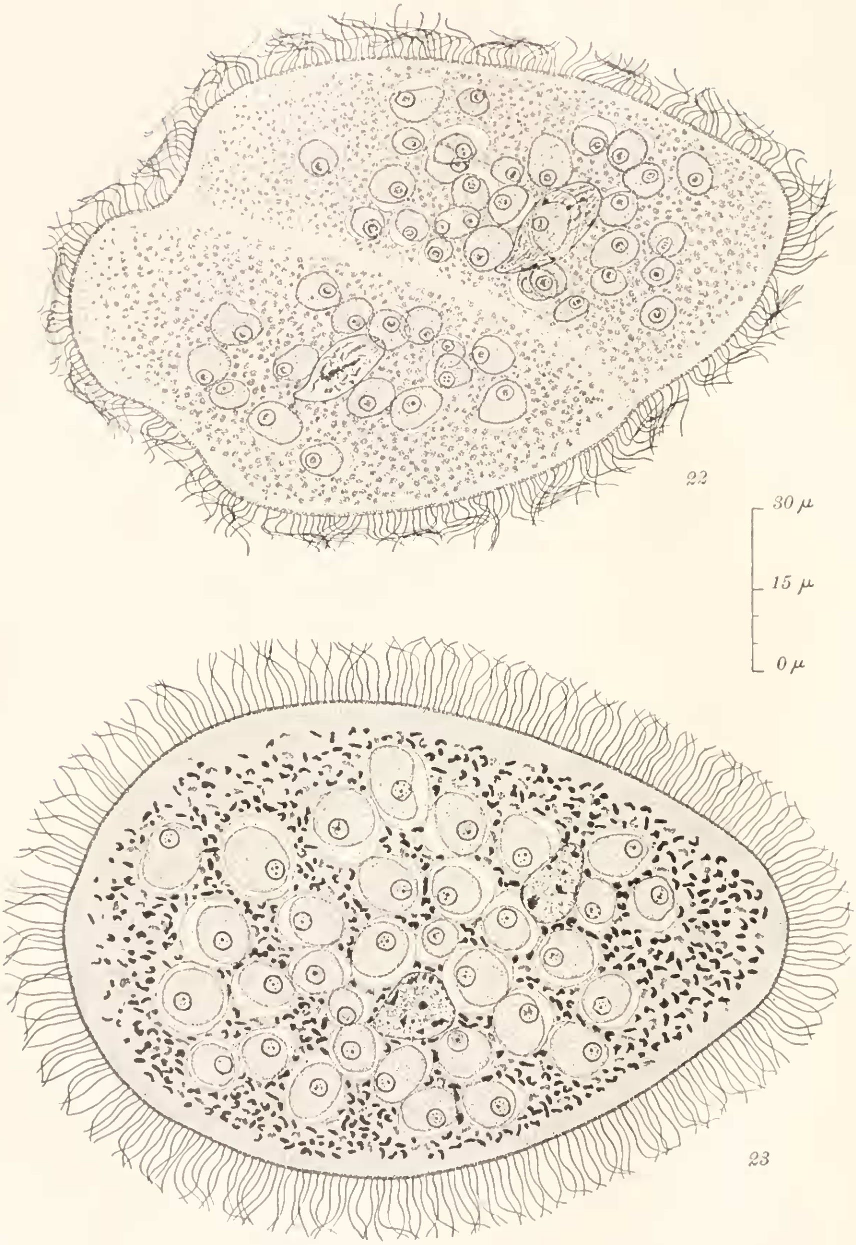


PLATE II

FIG. 22. A *Zelleriella* in a late stage of binary fission, containing many endamœbæ. Cilia diagrammatic.

FIG. 23. A *Zelleriella* fairly well filled with the trophozoites of the *Endamœba*. Cilia diagrammatic.

Although there may be these hundreds of amœbæ within the ciliates, relatively few are found free in the lumen of the rectum of the anuran host (Fig. 12). These latter are thought to represent amœbæ that have either escaped from, or been freed by the disintegration of the ciliates.

Trophozoites

The amœba is a fairly small one; seventy-five nearly spherical trophozoites taken at random from a number of different hosts range in diameter from 5.3 μ–14.3 μ, averaging 8.0 μ. Some ciliates contain enormous numbers of very small amœbæ only, while others harbor the large forms.

The amœbæ present typical *Endamœba* nuclei. In well-fixed material there is usually a fairly even ring of peripheral chromatin at the nuclear membrane (Figs. 7, 9, and 14). At times, however, this ring is broken into masses and blobs (Figs. 10, 11, and 12). A karyosome, which frequently appears as a discrete granule surrounded by an area of more lightly staining material (Figs. 7, 10, 11, and 14), shows particularly well when the stain has been differentiated in iron alum. After picric acid destaining this karyosome is usually less prominent and the surrounding material more deeply stained (Figs. 9 and 12). Occasionally two nuclei are found within the same amœba (Fig. 11).

A number of dividing stages have been observed in the trophozoites. However, only the later stages of mitosis have been recognized, an anaphase being the first stage of which we are certain (Fig. 1). In these later mitotic stages, in the mid-region of the elongating nucleus, there appears to be the progression of a series of granules from the nuclear membrane inward (Fig. 1). These granules do not appear to contribute to the formation of the chromosomes. As the daughter nuclei separate, this area of granules becomes larger (Figs. 2 and 3) and is finally stretched out as a "tail" from each of the telophase nuclei (Figs. 4 and 5). These granules finally disappear entirely and the reorganizing nucleus is featured by the layer of heavier peripheral chromatin on the one side of the membrane and the decidedly scarcer chromatin on the other (Fig. 6). This distribution of peripheral chromatin is typical of all the late telophase nuclei. It never entirely disappears from the nuclear membrane during mitosis. The exact chromosome number could not be determined from our material.

The question as to the food habits of these amœbæ is a difficult one to answer. They apparently feed on the endospherules of the opalinids, since these are the only inclusions noted in the trophozoites (Fig. 7). The apparently identical amœbæ found outside the ciliates contain no solid food.

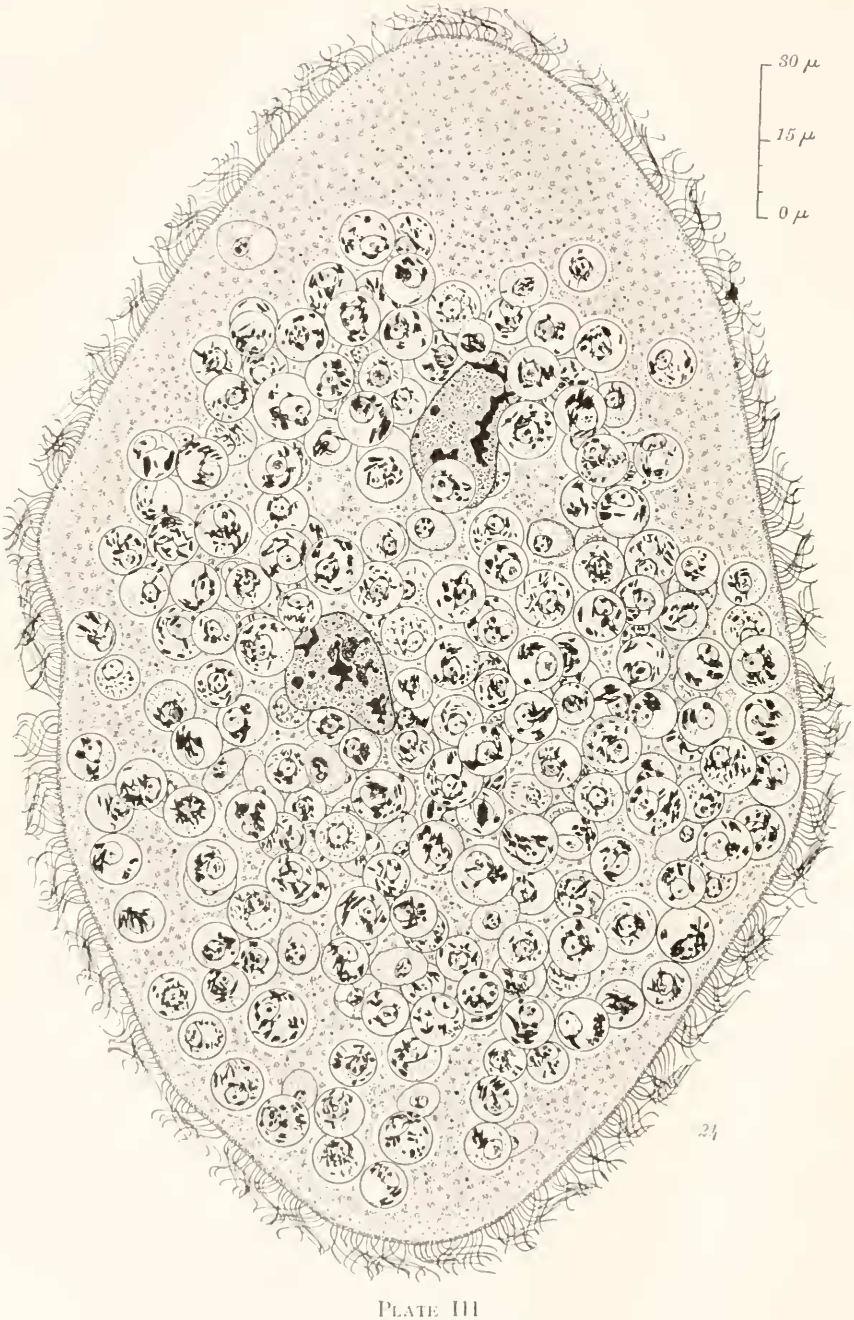


PLATE III

FIG. 24. *Zelleriella opisthocarya* containing over two hundred cysts of the *Endamœba*. Note uninucleate condition of the cysts, type of chromatoids, etc. Cilia diagrammatic.

Cysts

The cysts are less variable in size and slightly larger on the average than the trophozoites, though some trophozoites are larger than any cysts. Seventy-five cysts measured, range from 7.7 μ–10.8 μ, with an average diameter of 9.4 μ. Only the cysts within the ciliates were measured.

One of the most interesting facts concerning the cysts of this amœba is that, in our material, no single cyst in any ciliate ever contains more than one nucleus. In one opalinid, there are over two hundred cysts, all uninucleate (Fig. 24) and the only cases of more than one nucleus per cyst occur in those outside the ciliates (Figs. 17 and 18). Although division stages have been observed in the trophozoites, our material shows no cyst the nucleus of which is in any phase of mitosis.

TABLE I

Showing percentage infection of opalinids harboring the endamœbæ

Anuran host	Locality	Opalinid infected	Percentage infected in some individual anurans
Bufo cognatus cognatus	Flagstaff, Arizona	*Zelleriella hirsuta*	84
Bufo woodhousii	Near Tuba City, Arizona	*Zelleriella sp.*	19
Bufo woodhousii	Somerton, Arizona	*Zelleriella sp.*	22
Pleurodema bibroni	Near Coquimbo, Chile	*Zelleriella sp.*	91
Bufo marinus	Panama City, Panama	*Zelleriella opisthocarya*	51

The chromatoids of this amœba vary in appearance. Many are splinter-shaped, typical of *E. coli* (Figs. 15, 17, and 24), while others are bar-shaped, typical of *E. histolytica* (Fig. 18). Glycogen vacuoles are present in many of the cysts (Fig. 15).

Percentage Infection within Some Individual Anurans

Table I shows the percentage infection of opalinids harboring the endamœbæ. It is noted that in the 51 per cent infection of *Zelleriella opisthocarya* from *Bufo marinus* from Panama, only 4 per cent of the small and possibly precystic ciliates (averaging 60 μ in length) are parasitized, whereas among the large forms (averaging 230 μ in length), 98 per cent are infected. A possible explanation of this interesting phenomenon is available. It is a well-known fact that, previous to encystment, there is a period in which the opalinids divide very rapidly, giving rise to very small forms which later encyst. It is possible that,

TABLE II

Showing anuran hosts, localities, etc. for opalinids known to harbor endamœbæ

Anuran host	Locality	Describer	Opalinid infected
Polypedates maculatus . . .	Ceylon	Metcalf, 1923 (from Dobell's slide)	*Opalina virgula**
Bufo woodhousii	Utah	Metcalf, 1923	*Zelleriella sp.**
Bufo cognatus	Phoenix, Arizona	Metcalf, 1923	*Zelleriella hirsuta**
Paludicola signifera	City of São Paulo, Brazil	Carini, 1933	*Zelleriella brasiliensis*†
Engystoma ovale .	City of São Paulo, Brazil	Carini, 1933	*Zelleriella falcata*‡
Bufo marinus	Panama City, Panama	Stabler, 1933	*Zelleriella opisthocarya*
Bufo woodhousii	Somerton, Arizona	Stabler, 1933	*Zelleriella sp.*
Leptodactylus ocellatus . .	Uruguay	Carini and Reichenow, 1935	*Zelleriella brasiliensis*
Bufo cognatus cognatus . . .	Flagstaff, Arizona	Stabler and Chen, 1936	*Zelleriella hirsuta*
Bufo woodhousii	Near Tuba City, Arizona	Stabler and Chen, 1936	*Zelleriella sp.*
Pleurodema bibroni . .	Near Coquimbo, Chile	Stabler and Chen, 1936	*Zelleriella sp.*
Hyla nana	Angra dos Reis, State of Rio de Janeiro, Brazil	Stabler and Chen, 1936	*Zelleriella sp.*§
Bufo woodhousii	Utah	Stabler and Chen, 1936	*Opalina woodhousii*§

* Amœbæ described by Metcalf (1923) as "secondary" and degenerating nuclei.

† Changed by Carini and Reichenow (1935) to *Zelleriella paludicolæ*. Amœbæ described by Carini (1933) as *Brumptina brasiliensis*.

‡ Amœbæ described by Carini (1933) as *Brumptina paulista*.

§ From Metcalf's slides.

before encystment, the opalinids divide so fast that their rate of division is much more rapid than that of the amœbæ inside them. The large and parasitized zelleriellas which originally contain only a few amœbæ would produce, by successive divisions, a number of precystic forms, some with endamœbæ, others without.

Anuran Hosts Harboring Parasitized Opalinids and the Geographical Distribution of these Hosts

Metcalf (1923) figured *Opalina virgula* from *Polypedates maculatus* from Ceylon (Fig. 171*c*), a species of *Zelleriella* from *Bufo woodhousii* from Utah (Fig. 98*b*, *e* and *g*) and *Z. hirsuta* from *Bufo cognatus* from Arizona (Fig. 99*c*, *d* and *e;* Fig. 100*c*), containing what he called "secondary" and degenerating nuclei. The objects within those opalinids we now know, as the result of our study of Metcalf's original slides, to be identical with the endamœbæ described here. Carini (1933) described what he believed to be two species of organisms in zelleriellas from *Paludicola signifera* and *Engystoma ovale* from São Paulo, Brazil. These he was unable to identify and created for them the new genus *Brumptina*. These likewise prove to be identical with this *Endamœba* (Stabler, 1933; Carini and Reichenow, 1935). In a recent report on the subject, Carini and Reichenow (1935) have found the amœbæ in *Zelleriella brasiliensis* from *Leptodactylus ocellatus* from Uruguay. These, with the present authors' data, as shown in Table II, constitute all the observations that to our knowledge have been made on these amœbæ.

We have examined frogs and toads from such widely separated localities as Central and South America, Bermuda, the West Indies and a number of places in the United States. From some localities, large numbers of specimens of a given species of anuran have been examined. The same species of anuran host from different regions harbors opalinids infected with *Endamœba* in one locality and not in another. Then, too, from any particular region some individuals of a particular species of anuran contain parasitized opalinids, others do not.

Parasites of the Endamœbæ

In *Pleurodema bibroni* from Chile and *Bufo marinus* from Panama, some of the endamœbæ in the zelleriellas are parasitized by what appears to be a *Sphærita* (Fig. 13). These organisms are small and spherical, from one to fifty occurring in a single amœba. Occasionally they are found in the cytoplasm of the zelleriellas.

Discussion

It seems surprising that, with the exception of the short account by Stabler (1933) and the recent publication by Carini and Reichenow (1935), no other description of this organism has been published in which its true nature has been appreciated.

Carini and Reichenow are correct in believing Metcalf's *Zelleriella hirsuta* from *Bufo cognatus* to contain endamœbæ, but are mistaken in suggesting that the objects in *Zelleriella sp.* from *B. woodhousii* are *Sphærita*. They overlooked Metcalf's figure of the same bodies in *Opalina virgula* (Metcalf, 1923, Fig. 171*c*).

From our examination of Carini's preparations, we can agree with Carini and Reichenow's (1935) assertion that the *Zelleriella* from *Paludicola signifera* contains an *Endamœba*, an opinion recorded by Stabler in 1933. Carini (1933) probably failed to recognize these objects as amœbæ because of poor fixation and staining, but we have found undoubted trophozoites (Fig. 30) and cysts (Fig. 31) within the zelleriellas from his slides.

Unlike our own, Carini's preparations of opalinids from *P. signifera* show an abundance of uni-, bi- and quadri-nucleate cysts free from the ciliates (Figs. 32, 33, 34 and 35). As mentioned by Carini and Reichenow, these cysts present essentially the same morphology as the ones within the ciliates. We have observed, however, an additional feature of these cysts in Carini's preparations not noted by the above workers. The majority of the cysts outside the opalinids possess an enveloping membrane outside of and apart from the cyst wall (Figs. 32, 33, 34, and 35). This was also noted as a feature of *E. ranarum* by Sanders in 1931.

Concerning the trophozoites, Carini and Reichenow (1935) state that, with one exception, they were never found free from the ciliates. In Carini's own material from *P. signifera* the present writers have

EXPLANATION OF PLATE IV

FIG. 25. *Endamœba* trophozoite in *Zelleriella hirsuta* from *Bufo cognatus*, from Metcalf's slide.

FIG. 26. *Endamœba* trophozoite in *Zelleriella sp.* from *Bufo woodhousii*, from Metcalf's slide. Due probably to unsatisfactory technique, the *Endamœba* characteristics are not well shown in this animal.

FIG. 27. *Endamœba* trophozoite in *Opalina woodhousii* from *Bufo woodhousii*, from Metcalf's slide.

FIG. 28. *Endamœba* trophozoite in *Zelleriella sp.* from *Hyla nana*, from Metcalf's slide.

FIG. 29. *Endamœba* trophozoite in *Opalina virgula* from *Polypedates maculatus*, from Dobell's slide.

FIG. 30. *Endamœba* trophozoite in *Zelleriella paludicolæ* from *Paludicola signifera*, from Carini's slide.

FIG. 31. *Endamœba* cyst in *Zelleriella paludicolæ* from *Paludicola signifera*, from Carini's slide. Compare with Fig. 32.

FIG. 32. *Endamœba* cyst outside the ciliates from *Paludicola signifera*, from Carini's slide. Note the single nucleus and extracystic membrane. Compare with Fig. 31.

FIG. 33. *Endamœba* cyst outside the ciliates from *Paludicola signifera*, from Carini's slide. Note the four nuclei and extracystic membrane.

FIG. 34. Same as Fig. 33.

FIG. 35. *Endamœba* cyst outside the ciliates from *Paludicola signifera*, from Carini's slide. Note the two nuclei and extracystic membrane.

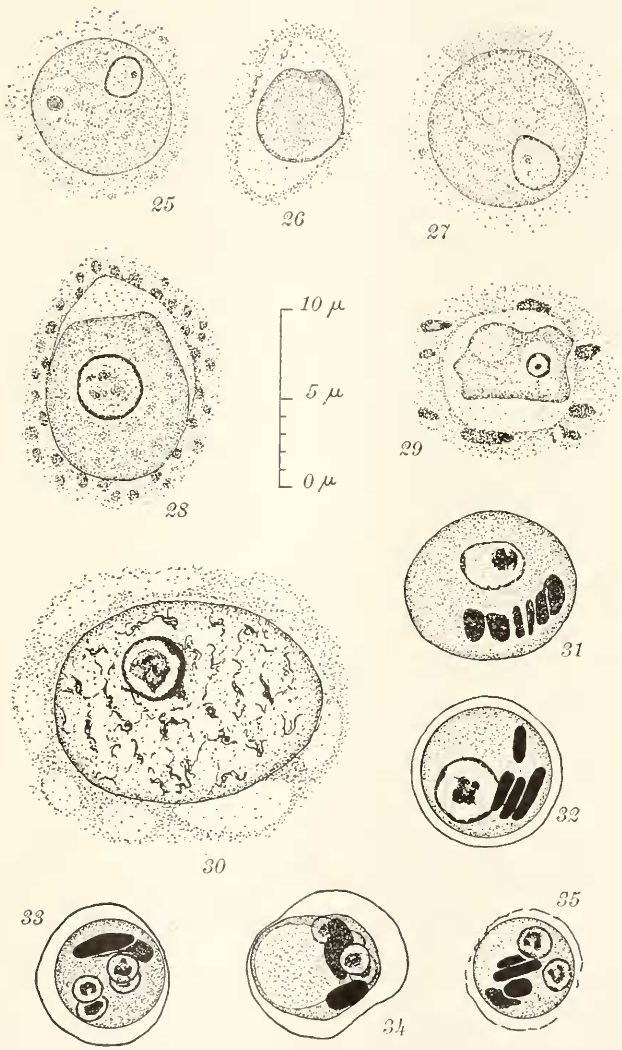


Plate IV

observed dozens of amœbæ outside the opalinids. We also cannot support Carini and Reichenow's assertion that no karyosomes can be seen in the nuclei of these amœbæ since this nuclear structure has been found in our material. Then, too, in our slides the amœbæ inside the ciliates are occasionally found filled with the endospherules of the latter, in contrast to the above writers' findings that they were always free of solid food.

Carini and Reichenow believed that, in their material, trophozoites of the amœba invade ciliates already containing the uninucleate cysts and then proceed to phagocytize these cysts. Their figure of this is unconvincing and we have seen nothing in any of Carini's nor in our own slides suggesting this phenomenon.

This brings us to the question of the specific name for this amœba. Carini and Reichenow believed it to be either identical with *E. ranarum* or a race or species derived from it. Stabler (1933) identified it as an *Endamœba*, a decision confirmed by Carini and Reichenow (1935). He gave it the specific name *E. brasiliensis*, since Carini (1933), who gave the first description of these organisms, has designated *B. brasiliensis* as the name of his first species of *Brumptina* (= *Endamœba*). It has since been recalled that Aragão in 1914 used the name *E. brasiliensis* for what he considered to be a new amœba from man. It is generally agreed that Aragão's description deals mainly with *Endamœba coli*, the name therefore being dismissed as a synonym of the latter form. *E. brasiliensis* cannot, then, be revived for the form described here and as Carini designated a second species of *Brumptina*, *B. paulista*, this species name becomes the one available. If this is a distinct species of amœba, its name should then be *Endamœba paulista* (Carini, 1933).

However, at the present time we do not attempt to establish its specific identity, because the question as to its synonymy with *E. ranarum* is still unanswered. It must be admitted that morphologically they are quite similar, presenting the extracystic membrane, rather similar chromatoids, etc. *E. ranarum*, however, is somewhat larger, most writers (Sanders, 1931) stating that it ranges from 10 μ–30 μ in diameter, while the range for the amœba from the opalinids is 5.3 μ–14.3 μ. Also the food of the latter organism seems to be different. *E. ranarum* feeds readily on bacteria and debris in the rectum of the anuran, while these amœbæ, outside the ciliates, are never observed to contain food and those in the ciliates contain ingested endospherules only. In all probability infection experiments will be necessary to finally establish its identity.

Still another question concerns the mode of entrance of the amœbæ into the astomatous opalinids. This process has never been observed by us. It seems probable that one or a few amœbæ may enter a ciliate and the progeny eventually fill the host. They may enter the ciliates when the rectal contents of the frogs and toads are rather dry, this condition preventing freedom of motion on the part of the ciliates. As it has been shown that the opalinids can divide successfully when containing amœbæ and that division stages have been observed in the amœbæ within the ciliates, these, too, may be important factors in carrying on the infection.

It seems possible that the cysts of the endamœbæ which have been freed from the ciliates constitute an infective stage so far as the new anuran host is concerned. They may be deposited in the ponds at the breeding season and be available for ingestion, along with the cysts of the opalinids, by the tadpoles. In the new anuran host we again face the problem of the penetration of the rapidly swimming ciliates by the slowly moving amœbæ. It is also possible that the amœbæ may even be within the encysted opalinids and pass from adult anurans to tadpoles with the latter.

Many other problems concerning this amœba remain yet to be solved. Why do some ciliates contain such large numbers of very small amœbæ and is there any significance to be attached to these small forms? Do the cysts always remain in the uninucleate condition until released from the ciliates? What are the appearances of the prophase and metaphase stages of mitosis? Would some races of undoubted *E. ranarum* invade opalinids?

Summary

1. Observations are presented on an *Endamœba* living in the opalinid ciliates from anuran hosts.

2. Large numbers of frogs and toads from widely separated regions have been examined. The endamœbæ have been found in some of the opalinids collected from the United States (Arizona and Utah), Panama (Panama City), Brazil (states of São Paulo and Rio de Janeiro), Chile (near Coquimbo), Uruguay and Ceylon. In a given locality, only certain species of anurans and a certain percentage of individuals of a given species contain the parasitized opalinids.

3. Within some individual anuran hosts, 91 per cent of the ciliates are infected. There may be a single, a few, many, or over a hundred of these amœbæ within a single opalinid. Usually in any one ciliate all the amœbæ are in the same stage of development, either cysts or trophozoites.

4. The amœbæ seem to produce no serious effect on the opalinids,

as the latter swim actively about in the saline solution and were seen to undergo binary fission even though heavily parasitized.

5. The trophozoites range in diameter from 5.3 μ–14.3 μ, averaging 8.0 μ. They appear to feed on the endospherules of the opalinids and a number of dividing stages have also been observed in the trophozoites.

6. The cysts are less variable in size and are slightly larger on the average (9.4 μ) than the trophozoites, though some trophozoites are larger than any cysts. Only uninucleate cysts have been found within the opalinids although uninucleate, binucleate and quadrinucleate ones were found outside the ciliates. The chromatoids vary in appearance; many are splinter-shaped, while others are bar-shaped. Glycogen vacuoles are present in many cysts.

7. In the zelleriellas from *Pleurodema bibroni* from Chile and in *Bufo marinus* from Panama, some of the endamœbæ were invaded by a *Sphærita*-like organism, which was also observed in the cytoplasm of some of the ciliates.

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